Swami Ramanand Teerth Marathwada University, Nanded, Maharashtra, India

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Antibiotic Resistance: A Comparative Genetic approach in *E.coli*.

Research Proposal Submitted
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Submitted by

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Abstract of proposed investigation:

The term stress accounts a change in conditions of relative environmental factors to a significant extent from organism most favourable optimal growth parameters. Microorganisms produce short and long term responses when exposed to such unfavourable challenging environmental conditions. Such responses are produced to what extent, what their generative potential is and how they transfer to move across their stress conditions to stabilise and survive in nature is still unclear. Use and overuse of different types of antibiotics against pathogenic bacteria leads the change by the bacteria towards the phenomenon of antibiotic resistance. In recent years study regarding antibiotics stress resistance is going on especially for *E.coli.* The whole genome DNA sequencing analysis has tremendous potential to study the different resistance-conferring genes. Genomics data can be utilized to find out different resistance genes for different types of antibiotics. However we can comparatively study responsible resistance genes their synthesized proteins with the approach of molecular simulations and dynamics.

Introduction:

Escherichia coli is a bacterium with a special place in the microbiological world since it can cause severe infections in humans and animals but also represents a significant part of the autochthonous microbiota of the different hosts. of major concern is a possible transmission of virulent and/or resistant *E.coli*between animals and humans through numerous pathways, *E.coli*also represents a major reservoir of resistance genes that may be responsible for treatmentfailures in both human and veterinary medicine. An increasing number of resistance genes have been identified in *E.coli*isolates during the last decades, and many of these resistance genes were acquired by horizontal gene transfer. In the enterobacterial gene pool, *E.coli*acts as a donor and as a recipient of resistance genes and thereby can acquire resistance genes from other bacteria but can also pass on its resistance genes to other bacteria. In general, antimicrobial resistance in *E.coli*is considered one of the major challenges in both humans and animals at a worldwide scale and needs to be considered as a real public health concern. (Laurent Poirel*et.al.*,2018)

The presence of Antimicrobial resistant genes harboured by bacteria can be determined by a variety of molecular methods, including PCR, DNA microarray and whole genome sequencing (WGS) (Anjum MF, 2015). Unlike PCR and microarrays, WGS offers the advantage of being able to screen the bacterial genome for multiple genes and mutations associated with Antimicrobial resistance, which can be used to predict phenotypic susceptibility to antimicrobials. (Ellington MJ et.al., 2017).

Review of Literature and Development in the subject:

The pathogenic *E.coli* isolates can be classified into different pathotypes or pathovars, where each pathotype causes a different disease. The intestinal pathogenic *E.coli*pathovars are responsible for disorders in the gut ranging from mild diarrhoea to severe colitis, while the extra-intestinal pathogenic *E.coli* pathovars are mostly asymptomatic inhabitants of the intestinal tract that cause extra-intestinal diseases after migrating to other parts of the body, such as the urinary tract or the blood stream. Animal diseases due to *E.coli*can also be caused by *E.coli*isolates originating from the environmental reservoir or other infected individuals. Pathogenic and non-pathogenic *E.coli* differ by the acquisition or loss of virulence-associated traits associated with *E.coli*pathogenicity. The number of genes present in the *E.coli* genome varies from 4,000 to 5,000 genes, with approximately 3,000 genes shared by the different isolates, whereas the others mostly correspond to colonization or virulence determinants. Advanced insights in the genomic plasticity of *E.coli* have been possible by the use of whole-genome sequencing, providing a better understanding of the core and accessory genomes of pathogenic and commensal *E.coli*isolates. (Laurent poirel et.al., 2018)

Multidrug-resistant *Enterobacteriaceae*are emerging as a serious infectious disease challenge. They can accumulate many antimicrobial resistance genes through horizontal transfer of genetic elements, those coding for b-lactamases and carbapenemases, fluoroquinolones and aminoglycosides being of particular concern (Ellington MJ et.al., 2017).

A small number of studies have assessed the feasibility of using WGS to infer AMR in *E.coli*and *Klebsiella pneumoniae*genomes; they are largely based on screening for known acquired AMR genes and a small number of known resistance-conferring mutations, such as those associated with ciprofloxacin resistance (Ellington MJ et.al., 2017).

In one study, (N.Stoesseret al., 2013) reported 95% concordance between phenotypic and WGS-predicted susceptibility for seven commonly used agents (amoxicillin, amoxicillin/clavulanic acid, ciprofloxacin, gentamicin, ceftriaxone, ceftazidime and meropenem) by querying 143 assembled genomes from *E.coli*and *K. pneumoniae*with a compiled database of acquired AMR sequences and mutations in the quinolone-resistance-determining regions of gyrAand parC.

Salinity was found to be the most important abiotic factor that re-shape Anbiotic Resistant Genes distribution profile in the salt-affected environment. In addition, the shift of Actinobacteria, an

important phylum of Anbiotic Resistant Genes carrier in natural ecosystems, is also likely responsible for the decrease of Anbiotic Resistant Genes. Furthermore, high salinity would promote resistant plasmids elimination by reducing the relative fitness of plasmid-harboring strains, as well as inhibit the horizontal transfer of plasmids (Lu Tan et.al., 2019).

WGS is a powerful tool for accurately predicting AMR that can be used for national surveillance purposes. Additionally, it can detect resistance genes from a wider panel of antimicrobials whose phenotypes are currently not monitored but may be of importance in the future (Stubberfield E et.al., 2018).

The adoption of routine use of whole-genome sequencing in clinical microbiology would be a revolution in clinical medicine. While bacterial pathogens, there is also progress being made to use WGS to predict drug resistance in viruses, fungi, and eukaryotic parasites. The major barriers to adoption for routine use stem from technological limitations that make the process currently too slow and expensive and the need to demonstrate the advantages of a new approach to diagnosis for sceptical end-users in clinical microbiology laboratories. Along with the development of instrumentation, there is need for efficient data management of WGS data, user-friendly pipelines that do not require specialized bioinformaticians for use, and rapidly interpretable results (SuM et.al., 2019).

Objectives of Research/ Proposed Hypothesis:

Purpose of the study is to identify antibiotic stress resistant *E.coli* strain and probable mode of action of newly synthesised proteins by molecular dynamics studies, whole genome sequencing and mapping of stress/antibiotic resistant genes, identification of coding regions, prediction and validation of protein structures.

The main objectives of the study are as the following:

- Isolation of antibiotic resistant strains of *E.coli*.
- Whole genome sequencing of isolated strain.
- Mapping of possibly newly synthesized proteins as compare to standard strain.
- Mapping of protein sequences, identifying earlier strain.
- Prediction of protein structure
- Validation of Antibiotic resistance by molecular simulations and molecular dynamics.

Methodology to be adopted:

- Sample Collection:Identified well characterised strain will be procured from major repositories from India like MTCC, Chandigarh or NCCS, Pune.

- Isolation of multidrug resistant *E.coli* by using standard protocol.
- Screening of antibiotic resistance strains of *E.coli*
- Bacterial identification will be done by biochemical tests.
- DNA Extraction from identified antibiotic resistant *E.coli* strain will be carried out by using standard methods.
- DNA Sequencing will be done using high throughput methods like Illumine Sequencing, for the extracted DNA from identified strain earlier step.
- Mapping of sequences of new synthesized proteins responsible for antibiotic resistance or synthesised in response to antibioticwill be done using major database like NCBI Genome Workbench and other tools available online or tools.
- Prediction and validation of synthesized proteins by molecular simulations and dynamics methods.

Importance of study/ Society application:

- Validated structures of protein will help to find out active site & potential inhibitor to stop/retard the growth of growth of organism.

- Understanding more about these mechanisms should hopefully lead to better treatment options for infective diseases, and development of antimicrobial drugs that can withstand the microorganism's attempts to become resistant.

- De novo or Molecular dynamics study of antibiotic resistance mechanisms can be studied for detoxification of the antibiotic molecule or mutations in the designated target.

National Status:

Ashish kumarsingh and his group in June 2018 from Department of Microbiology, School of Life Sciences, Sikkim University, Gangtok, Sikkim, India has studied a close association between different demographic factors and the high prevalence of antibiotic-resistant commensal *E. coli* in their study they suggests a concern over rising misuse of antibiotics that warrants a future threat of emergence of multidrug-resistant pathogen isolates.

Asis Khan and his team from National Institute of Cholera and Enteric Diseases, Beliaghata, worked on Antibiotic resistance, virulence gene, and molecular profiles of Shiga toxin-producing Escherichia coli (STEC) non-O157 strains isolated from human stool samples, cow stool samples, and beef samples over a period of 2 years in Calcutta, India, were determined.

RutujaDhawde and her team from The Foundation for Medical Research, 84A, R.G. Thadani Marg, Worli, Mumbai 400 018 studied ceftazidime- and ciprofloxacin-resistant—or dual drug-resistant (DDR)—E. coli were isolated from river Mula-Mutha, which flows through rural Pune district and Pune city. The DDR E. coli were further examined for antibiotic resistance to six additional antibiotics. The study also included detection of genes responsible for ceftazidime and ciprofloxacin resistance and vectors for horizontal gene transfer results of study showed that results showed that antibiotic resistance genes (ARGs) and integrons were present in the E. coli isolated from the river at points adjoining and downstream of Pune city.

Vishal Diwan and team members from Department of Public Health and Environment, R.D. Gardi Medical College, Ujjain has studied to characterize the seasonal variation, over one year, in water-quality, antibiotic residue levels, antibiotic resistance genes and antibiotic resistance in Escherichia

coli isolates from water and sediment of the Kshipra River in Central India. And found that In river water, seasonal variation was significantly associated with various water quality parameters, presence of sulfamethoxazole residues, bacteria resistant to ampicillin, cefepime, meropenem, amikacin, gentamicin, tigecycline, multidrug resistance and CTX-M-1 gene. The majority of the Extended Spectrum Beta-Lactamase (ESBL)-producing E. coli isolates from river water and sediment in all different seasons belonged to phylogenetic group A or B1.

Atul Kothari and Vishal Sagar from Department of Microbiology, Max Heart & Vascular Institute studied Press Enclave Road, Saket, Delhi, India studied on Antibiotic resistance in pathogens causing community-acquired urinary tract infections in India and concluded that High levels of ESBL producers among gram negative CA-uropathogens was seen in our country. This, along with the alarming rate of resistance to ciprofloxacin, SXT and amoxicillin, precludes the use of these commonly used antibiotics for empiric treatment of community-acquired urinary tract infections in India.

International status:

Emmanuel Nji and group from BioStruct-Africa, Vårby, 143 43 Stockholm, Sweden in 2021 reported the study on High prevalence of antibiotic resistance in commensal Escherichia coli from healthy human sources in community settings and concluded that , commensal E. coli is the main reservoir for spreading antibiotic resistance to other pathogenic enteric bacteria via mobile genetic elements. Significance of the study

Umji choi and changro lee in 2019 from Department of Biological Sciences and Bioinformatics, Myongji University, Yongin, South Korea studied on Distinct Roles of Outer Membrane Porins in Antibiotic Resistance and Membrane Integrity in *Escherichia coli.*

Marion Hutinel and team Centre for Antibiotic Resistance Research (CARe), University of Gothenburg, Gothenburg, Sweden studied on Population-level surveillance of antibiotic resistance in Escherichia coli through sewage analysis their conclusion provide support for development of a low-cost, sewage-based surveillance system for antibiotic resistance in E. coli, which could complement current monitoring systems and provide clinically relevant antibiotic resistance data for countries and regions where surveillance is lacking.

Jinling Shen and team worked on Prevalence, Antimicrobial Resistance, and Whole Genome Sequencing Analysis of Shiga Toxin-Producing Escherichia coli (STEC) and Enteropathogenic Escherichia coli (EPEC) from Imported Foods in China during 2015–2021 and their results revealed that s revealed that food can act as a reservoir of STEC/EPEC with pathogenic potential, and had the potential ability to transfer antibiotic resistance and virulence genes.

LicaLozica and team studied on Genomic Analysis of Escherichia coli Longitudinally Isolated from Broiler Breeder Flocks after the Application of an Autogenous Vaccine and concluded that the frequency of AMR genes showed a decreasing trend over time in both farms. The highest prevalence was detected in strains belonging to the B1 phylogenetic group, confirming the previous notion that commensal strains act as reservoirs and carry more resistance genes than pathogenic strains that are mostly associated with virulence genes.

Proposed work Plan/ Formulation and Structure of Study:

Following steps will be employed to carry out proposed research work:

- Sample collection
- Isolation of antibiotic resistant strains of *E.coli* on agar plates and identification
- Biochemical tests for confirmation of bacterial strain.
- Screening of Antibiotic Resistance *E.coli*
- DNA extraction of Antibiotic resistant *E coli*
- DNA sequencing of isolated strain.
- Bioinformatics work will be carried out followed by sequencing i.e. Mapping of possibly newly synthesized proteins as compare to standard strain.
- Mapping of protein sequences, identifying earlier strain.
- Prediction of protein structure validation of antibiotic resistance by molecular simulations and molecular dynamic methods.

Year-wise Plan of work and targets to be achieved

I year

- Course work and review of literatures.

II year

- Isolation of *E. coli* strains and whole genome sequencing of isolated strain.

III year

- Mapping of newly synthesized proteins as compare to standard protein
- Mapping of sequences and identifying strain.
- Prediction of protein structure and validation of antibiotic resistance by molecular dynamics.

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